C-reactive protein and NT-proBNP as surrogate markers for pulmonary hypertension in Gaucher disease

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Abstract

Background. N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) values correlate with mild-moderate pulmonary hypertension assessed by tricuspid insufficiency (TI) gradient \(\geq 30\) mm Hg in Gaucher disease. The purpose of this study is to ascertain improved risk stratification based on correlation with NT-proBNP and C-reactive protein (CRP), a standard marker of inflammation.

Methods. Patients with type I Gaucher disease were selected to reflect differing degrees of echocardiographically determined TI gradient values. NT-proBNP was performed by immunoassay and CRP by standard methods.

Findings. There were 45 patients (18 males; 40%); mean age = 42.5 (range: 4–80) years. Median NT-proBNP value = 153 (range: 46–6703) pg/ml; median CRP value = 0.145 (range: 0.02–2.69) mg/dl. There was a statistically significant correlation between these values (\(r = 0.445, P < 0.01\)). Elevations of CRP and NT-proBNP were risk factors for pulmonary hypertension with odds ratios of 8.47 and 4.9, respectively. The area under the Receiving Operator Characteristic (ROC) curve for diagnosis of pulmonary hypertension was 0.93 (0.04 for CRP, and 0.7 (0.1 for NT-proBNP. All patients with pulmonary hypertension had elevation of either CRP or NT-proBNP (100% sensitivity).

Conclusions. Elevated CRP was a better predictor of pulmonary hypertension in Gaucher disease than elevated NT-proBNP values. Elevated CRP (>0.5 mg/dl) or elevated NT-proBNP (>150 pg/ml) reduces the need to perform echocardiography by more than half, even in this group with over-representation of pulmonary hypertension. The role of inflammatory features in pulmonary hypertension in Gaucher disease is discussed. Further studies are required to ascertain if this approach is useful for prognosis of pulmonary hypertension.

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Keywords: Gaucher disease; Pulmonary hypertension; NT-proBNP; CRP; Tricuspid insufficiency; Inflammatory markers

Introduction

Primary pulmonary hypertension is a rare life-threatening disorder characterized by elevated pulmonary artery pressure whose etiology is incompletely understood; current therapies mitigate signs and symptoms, but progression to right heart failure is only slowed rather than reversed. Echocardiography is currently the preferred test for indirect evaluation of pulmonary hypertension, whether secondary or primary [1]. Recently, the use of the N-terminal fragment of the pro-hormone of brain natriuretic peptide (NT-proBNP) has been shown to be effective for diagnosis and prognosis in several cardiovascular disorders [2] and in symptomatic primary pulmonary hypertension [3].

Gaucher disease is caused by an enzymatic defect with accumulation of glucocerebroside in the cells of the monocyte–macrophage system. The presenting signs of systemic involvement generally include hepatosplenomegaly and hypersplenism. Infiltrative lung involvement and/or pulmonary hypertension secondary to severe systemic disease are
increasingly recognized in both the neuronopathic and non-
neuronopathic types of Gaucher disease [4].

Enzyme replacement therapy ameliorates most symp-
toms and signs including secondary lung involvement and
pulmonary hypertension, but has also been implicated in
inducing a primary-like form of pulmonary hypertension in
a few patients [5]. The practice to routinely monitor
tricuspid insufficiency (TI) gradient values by echocardiog-
raphy has resulted in identifying patients with “incipient”
pulmonary hypertension when TI gradient values are
 elevated beyond 30 mm Hg [6]. Often, withdrawal from
enzyme therapy will halt the progression to overt symptoms
and signs of severe pulmonary hypertension.

Recently, in a pilot study by our group, NT-proBNP
values were seen to correlate with TI gradient values in
a cohort of patients with Gaucher disease who were selected to
represent a spectrum of TI gradient values [7]. Neverthe-
less, some patients with elevated TI gradients (≥30 mm Hg)
and elevated NT-proBNP values (>200 pg/ml) did not have
overt signs or symptoms of pulmonary hypertension.
In another context, when attempting to correlate laboratory
markers with specific organ severity/involvement in Gau-
cher disease such as bone and lung disease, we have shown
a correlation between d-dimer values and TI gradient values
[8] in an unselected cohort of adult patients. Our posited
hypothesis was that elevated d-dimer values are suggestive
of microthrombi. Yet, one may also entertain another
tenable hypothesis: that d-dimer elevations are indicative of
an inflammatory pathology.

Therefore, in attempting to more adequately assess
patients with Gaucher disease who may be at risk for
developing clinically significant pulmonary hypertension, a
multi-marker approach was considered. In the current pilot
study, high-sensitivity C-reactive protein (CRP) values were
used as a more sensitive measure of inflammation along
with d-dimer values, and NT-proBNP values as prognostic
in pulmonary hypertension, and these were serially com-
pared with TI gradient to ascertain whether laboratory
measures may be sufficiently predictive of patients who may
develop symptomatic pulmonary hypertension in Gaucher
disease.

**Methods**

There were 48 patients from a cohort of 199 patients for
whom there was long-term follow-up by echocardiography
and from whom sera had been taken. The sample cohort was
selected in order that there be a spectrum of echocardiog-
graphic findings. All 12 patients with TI gradients ≥30 mm
Hg at the time of blood sampling with simultaneous
evaluation of d-dimers and CRP during the 6-month period
between October 2003 and March 2004 were included.
Additional 36 patients who had been seen during this period
were chosen because their TI gradients spanned the range
10–29 mm Hg.

TI gradient values for these patients at the time of sam-
ple collection were “normal” (<30 mm Hg) or elevated
(≥30 mm Hg). There was no attempt to select for any other
criteria including sex, age, genotype, spleen status, disease
severity, or treatment status.

The NT-proBNP assay was performed as duplicates
using the electrochemoluminescence immunoassay (Elecsys
system 1010/2010 for the proBNP kit; Roche, Mannheim,
Germany). Elecsys proBNP contains polyclonal antibodies
that recognize epitopes located in the N-terminal part [1–76]
of proBNP (1–108). The assay is unaffected by icterus
(bilirubin < 35 mg/dl), hemolysis (hemoglobin < 1.4 g/dl),
or lipidemia (triglycerides < 4000 mg/dl). No cross-
reactivity (<0.001%) was observed with atrial natriuretic
peptide (ANP), NT-proANP, BNP, C-type natriuretic pep-
tide (CNP), adreomedullin, aldosterone, angiotensin I, II,
III, endothelin, renin, urodilatin, or Arg-vasopressin.

CRP was routinely obtained using a Boering BN II
Nephelometer (DADE Boering, Marburg, Germany) [9].

D-dimer levels were measured using the Miniquant d-
dimer assay (Biopool International, Ventura, CA, USA),
following manufacturer’s instructions.

TI gradient was obtained echocardiographically, measur-
ing the Doppler-derived right ventricle to right atrial
pressure gradient. Assuming right atrial pressure in the
range of 8–10 mm Hg, the TI gradient estimates right
ventricular systolic pressure, and in the absence of pul-
monary stenosis, systolic pulmonary artery pressure. Thus, a
TI gradient of 20 mm Hg corresponds to pulmonary artery
systolic pressure of >30 mm Hg.

This study was approved by the institutional Ethics
(Helsinki) Committee.

**Statistical analysis**

Data were expressed as medians and ranges or as
means. As most of the quantitative variables were not
normally distributed, comparison between groups was
performed using the non-parametric Mann–Whitney rank
sum test. The Spearman non-parametric correlation coef-
ficient was used to estimate the association between pairs
of variables. The Pearson correlation coefficient was
calculated only between the log transformation of the
NT-proNP and CRP levels. NT-proBNP and CRP levels
were dichotomized into normal and high levels (NT-
proBNP >200 pg/ml and CRP >0.5 mg/dl were considered
high). The sensitivity and specificity of these dichotomized
variables were calculated as predictors for pulmonary
hypertension (TI gradient ≥30 mm Hg).

The logistic regression model was applied in order to test
the simultaneous affect of both CRP and NT-proBNP levels
(dichotomized) on pulmonary hypertension. ROC curves
were generated using the SPSS software.

All statistical tests applied were two tailed, and a P value
of 5% or less was considered statistically significant.
Results

Of the original 48 patients, samples from 3 patients with TI gradients < 30 mm Hg were missing more than one lab value and therefore eliminated. The demographic characteristics of the remaining patients and the results of the assays are presented in Table 1. There was a statistically significant correlation (Spearman non-parametric correlation coefficient) between NT-proBNP and CRP ($r = 0.445, P < 0.01$).

Of the 16 patients with elevated NT-proBNP (>200 pg/ml), eight patients (50.0%) had normal CRP values (<0.5 mg/dl), three of these patients had an elevated TI gradient.

Of the 11 patients with elevated CRP, three patients (27.2%) had normal NT-proBNP values, two of these patients had elevated TI gradients.

Of the eight patients with elevated NT-proBNP and elevated CRP, 6 patients have pulmonary hypertension (75%) five of whom receive enzyme therapy.

Of the two patients with normal NT-proBNP and normal CRP, both had been withdrawn from enzyme treatment because of pulmonary hypertension; one patient was subsequently treated with a prostacyclin analogue.

To exclude the possibility that both markers are elevated because of enzyme therapy, we compared their values in patients receiving ($n = 28$) and not receiving ($n = 17$) enzyme replacement therapy. There was no difference between the groups (median NT-proBNP: enzyme-treated 157 pg/ml, no therapy 140 pg/ml, $P = 0.42$; median CRP: enzyme-treated 0.17, no therapy 0.14, $P = 0.67$).

When calculating the Spearman non-parametric correlation coefficient between d-dimer values and both NT-proBNP and CRP was 0.7 ($P < 0.01$) and 0.345 ($P = 0.05$), respectively. When calculating the Spearman non-parametric correlation coefficient between NT-proBNP and TI gradient ($N = 45$), there was a statistically significant correlation ($r = 0.54; P$ value $< 0.01$). Similarly, when calculating the Spearman non-parametric correlation coefficient between CRP and TI gradient, there was a statistically significant correlation ($N = 44$; correlation coefficient = 0.493; $P$ value = 0.001).

The sensitivity of the dichotomized NT-pro-BNP variable to predict pulmonary hypertension was 69.2%, and the specificity was 78.1%. The sensitivity of the dichotomized CRP variable to predict pulmonary hypertension was 61.5% and the specificity was 90.3%.

When the logistic regression model was applied to the data in order to test the simultaneous effect of both CRP and NT-proBNP levels (dichotomized) on pulmonary hypertension, CRP was found to be statistically significant ($P = 0.017$) and NT-proBNP yielded a borderline significance level ($P = 0.06$). The adjusted Odds Ratio for CRP was 8.47 (95% CI 1.464–50, i.e., very high variability), meaning that the odds of a person to develop pulmonary hypertension is 8.47 times greater if he has high CRP levels as compared to low CRP levels. The adjusted Odds Ratio for NT-proBNP was 4.9 (95% CI 0.937–25.64). The model predicted correctly 93% of the patients who do not have pulmonary hypertension (corresponding to a specificity level of 93%).

In receiver operating characteristics (ROC) analysis, the AUC (area under the curve) value for CRP for the diagnosis of pulmonary hypertension was $0.93 \pm 0.04$ (0.85–1.00), and for NT-proBNP was $0.7 \pm 0.1$ (0.51–0.9) as shown in Figs. 1a and b.

Because we had recommended the use of 200 pg/ml as the cut-off for NT-proBNP values in our previous study [7] but the manufacturer suggests 150 pg/ml as the upper limit of the normal range for non-elderly in its brochure, we applied the latter value to our analysis as well. Using NT-proBNP cut-off value of 150 pg/ml, the sensitivity was 100% and specificity 57% for the combination of NT-proBNP and CRP.

For NT-proBNP alone, using the cut-off value of 150 pg/ml, sensitivity was 78% and specificity was 56%.

Discussion

A multi-marker approach would be attractive to enhance identification of patients at risk for pulmonary hypertension, particularly for the purpose of early intervention. Whereas routine echocardiography has resulted in more accurate documentation of trends towards elevated TI gradients and hence has allowed for withdrawal of enzyme therapy in those patients in whom this may be therapeutic [5], TI gradients alone are not sufficiently accurate for risk stratification. Since enzyme replacement therapy is the gold standard of care, medical management of pulmonary hypertension

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Table 1

Demographic characteristics and results of NT-proBNP and CRP values

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NT-proBNP ≥ 30 mm Hg</th>
<th>NT-proBNP &lt; 30 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>7/12 (58%)</td>
<td>12/33 (36.4%)</td>
</tr>
<tr>
<td>Duration (range)</td>
<td>58.7 (38–80) years</td>
<td>36.6 (4–78) years</td>
</tr>
<tr>
<td>Enzyme treated</td>
<td>8/12 (66.7%)</td>
<td>20/33 (60.6%)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>274 (73–6703)</td>
<td>122 (466–1088)</td>
</tr>
<tr>
<td>NT-proBNP (pg/ml)</td>
<td>8/12 (66.7%)</td>
<td>8/33 (24.2%)</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>1.31 (0.12–2.69)</td>
<td>0.07 (0.02–0.65)</td>
</tr>
<tr>
<td>CRP &gt;0.5 mg/dl</td>
<td>9/12 (75%)</td>
<td>2/32* (6.25%)</td>
</tr>
<tr>
<td>Normal CRP</td>
<td>0/9 (0%)</td>
<td>6/7 (85.7%)</td>
</tr>
<tr>
<td>Elevated CRP/Normal CRP</td>
<td>1/9 (11.1%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>Normal CRP</td>
<td>7/10 (70%)</td>
<td>1/7 (14.3%)</td>
</tr>
<tr>
<td>NT-proBNP &lt; 0.5 mg/dl</td>
<td>2/12 (16.7%)</td>
<td>24/32* (75%)</td>
</tr>
</tbody>
</table>

* One patient had no data for CRP.

* Both patients had symptomatic pulmonary hypertension, both have been withdrawn from enzyme therapy and one of these patients has been receiving prostacyclin therapy (see text).
putatively induced by enzyme treatment is fraught with concern. Echocardiography, while recommended by us for routine monitoring of TI gradients, is nonetheless a scarce resource that is expensive to employ routinely; the results are operator-dependent, and not in all individuals is it possible to measure a TI gradient. Thus, there are practical concerns that may make a simple set of laboratory measures preferable as an early warning system.

Pulmonary hypertension may be life threatening. Therefore, NT-proBNP has been proposed as a potential marker for diagnosis and prognosis of pulmonary hypertension associated with other diseases. However, among patients with Gaucher disease when using TI gradients (≥30 mm Hg) and NT-proBNP (>200 pg/ml) results to predict pulmonary hypertension, there were some false-positives. Thus, it is of interest that the addition of CRP values, an inexpensive and routinely available test, enhances risk stratification. The addition of d-dimer values was not equally predictive of pulmonary hypertension as either CRP or NT-proBNP. We assume that this is because of the use of non-randomly chosen patients where there is a greater preponderance of elevated TI gradients relative to unselected cohorts [8].

There were two patients with elevated CRP, normal NT-proBNP and elevated TI gradients with symptomatic pulmonary hypertension. These patients had been withdrawn from enzyme replacement therapy because of pulmonary hypertension more than 3 years previously; one patient has been receiving inhaled prostacyclin analogue for more than two years, and hence was expected to have (and indeed, did have) normalization of NT-proBNP along with stabilization of TI gradient at about 50 mm Hg [7]. Therefore, in the current study, it is important to note that the lack of correlation between treatment status and either NT-proBNP or CRP precludes the conclusion that ERT per se is correlated with increases in any of these measures.

In reviewing the current data and despite our previous recommendation to use 200 pg/ml as the cut-off point for the normal range of NT-proBNP, post hoc analysis of NT-proBNP values using a normal range cut-off of 150 pg/ml as recommended by the manufacturer allowed us to achieve 100% sensitivity for negative prediction. We therefore now suggest that normal CRP values and/or normal NT-proBNP values (<150 pg/ml) for the purpose of an initial screening are valuable, allowing echocardiography to remain in abeyance unless one of these values becomes abnormal. Similarly, in a patient with elevated CRP or NT-proBNP values together with an elevated TI gradient, right heart catheterization, a non-trivial decision in patients with “incipient” pulmonary hypertension, would be based on early risk stratification.

Admittedly, because patients with Gaucher disease in our clinic are monitored every 6 months and in those in whom TI gradients are noted to increase, enzyme therapy is withdrawn, our clinic does not have a population in whom there are continuous increments in TI gradients.

NT-proBNP is used for both diagnosis and prognosis in other cardiac diseases and in pulmonary hypertension, so the further refinement of considering routinely available CRP values in addition to NT-proBNP evaluation may prove to be valuable particularly in pulmonary hypertension due to other etiologies and with an inflammatory component, such as sarcoidosis or scleroderma.

The hypothesis for this project arose from the appreciation of an underlying inflammatory profile in patients with Gaucher disease [10] that may be reflected in predisposition to symptomatic expression. Since inflammatory mechanisms appear to play a significant role in some types of pulmonary hypertension, and since elevated CRP levels are considered markers of the acute phase response and characterize progression of vascular injury, the choice of evaluating CRP values seemed tenable. It was however quite unexpected that CRP values alone were more predictive of elevated TI gradients and pulmonary hypertension than NT-proBNP values. Thus, the hypothesis of inflammation as an inimical process in Gaucher disease may be strengthened.

In conclusion, the results of the current pilot study in which two independent markers are compared with a non-invasive test for the purpose of risk identification for pulmonary hypertension, highlight the utility of a multi-

Fig. 1. (a and b) ROC curves for CRP and NT-proBNP, respectively. CI for ROC AUC (area under the curve): For CRP the 5 and 95 percentiles are 0.856–1.00; For NT-proBNP, 0.51–0.90.
factorial approach to this life-threatening complication. For patients who have elevated TI gradients but normal NT-proBNP and normal CRP, right heart catheterization (or other invasive procedures) may be withheld. When there is discordance between the two laboratory markers but in the presence of elevated TI gradient, the patient should still be monitored, albeit closely, before ascribing worsening pulmonary status. While in patients with low NT-proBNP and low CRP, there is apparently minimal risk of developing pulmonary hypertension, in patients with Gaucher disease with either elevated NT-proBNP or CRP values, the index of suspicion is raised for clinically significant pulmonary hypertension and medical management should therefore reflect this. Finally, the inclusion of an inflammatory marker (CRP) in the prognostic triad for pulmonary hypertension in Gaucher disease raises questions as to the role of inflammation, whether as an underlying chronic condition or as a subacute component, in either or both of these disease entities.

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References
